SYNOPSIS OF THE THESIS ENTITLED

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR ESTIMATION OF CARDIOVASCULAR AND ANTIDIABETIC DRUGS IN PHARMACEUTICAL FORMULATIONS

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Introduction

Analytical method development[1] plays a great role in the formulation and development of pharmaceutical drug products. Analytical method validation is required by analysts in the pharmaceutical industry, because adequately validated methods are essential for approvable regulatory filings.

More number of drug products are introduced into the market & the number is increasing every year. Analytical method development can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods.

The developed & validated analytical methods are used by quality control laboratories to ensure the identity, purity, potency and performance of formulated drug products. The effective single dose (having more efficacy) or combination therapy now days are becoming more popular, and needs a special quality control check. Therefore researchers are required to develop a new method for their analysis.

The analytical methods developed for the assessment of quality control of pharmaceutical products are always subjected to different requirements. The current good manufacturing practices (cGMP) regulation[2] requires the test methods that are used for assessing compliance of pharmaceutical product with established specification need proper accuracy and reliability. Therefore it is obligatory for the analytical researcher to adopt novel techniques that not only yield accurate and precise results but also are rapid and reproducible.

The various methods developed are used in quantitative analysis[3]. Amongst these the instrumental methods are important in quantitative and qualitative analysis due to their better sensitivity and rapidity. The overwhelming demand for better sensitivity, greater resolution, and automatic recording of data has resulted in more and more sophisticated and computerized instruments using which researcher can develop accurate, rapid, sensitive, economical, specific and reproducible methods.

Recent progress in method development has been largely a result of improvements in analytical instrumentation. The instrumental methods like spectrophotometry, high performance liquid chromatography (HPLC)[4] and high performance thin layer chromatography (HPTLC) [5] have better acceptance due to rapid speed of analysis.

The UV spectroscopic methods[6] which usually measures the intensity of electromagnetic radiation emitted or absorbed by the drugs is of great importance in the quality control test. The careful manipulations of the experimental parameters in UV-visible spectrometer often enables the analyst to estimate one or simultaneously, more than one

component present in a mixture without their prior separation. Spectroscopic methods using hydrotropic[7] solution (Solution of urea, Sodium acetate, Sodium citrate, Sodium benzoate, etc) now a day is also used for the determination of poorly water soluble drugs.

HPLC and HPTLC are the widely used techniques in the qualitative and quantitative analysis. HPLC is the most popular chromatographic technique used for the quality control of pharmaceuticals. The reason for the popularity of this method is its sensitivity and acceptability to accurately quantitative determinations and its suitability for separating non volatile species or even thermally fragile ones. Isocratic and gradient reverse-phase high-performance liquid chromatography (HPLC) have evolved as the primary techniques for the analysis of non-volatile active pharmaceutical ingredients and impurities. Above its entire wide spread applicability to substances that are of prime interest of industry to many fields of sciences and to the society.

HPTLC is the most reliable method amongst all for the quantitation at micro, nano and even when present in complex formulations. HPTLC can simultaneously handle several samples even of divergent nature and composition. The HPTLC technique is based on in situ measurement of visible, UV-absorbance, fluorescence or fluorescence quenching directly on the layer. Since chromatographic zones re-emit a lower light intensity than environment around it, the measurements are made comparison of reflection of spot with free area around it. The scanner converts barrel into peaks and peak height or area is related to the concentration of substance on the spot.

Validation of analytical method is the process of providing (through scientific studies) that an analytical method is acceptable for its intended use. Method validation provides the method development extremely Specific, Sensitive, linear, Precise and Accurate.

Stability indicating assay method may be defined as validated, quantitated analytical method, which can detect the chemical and physical change of drug substances and drug products with time which is specific so that the content of active ingredients, degradation products and the other component of interest can be determined accurately without interference. Related substances method is used to determine impurities & degradation products. A summation of assay value and impurity levels generally may be used to obtain mass balance for the test sample. The mass balance need not add to exactly 100 percent because of the analytical error associated with each analytical procedure. However the Mass Balance[8] may be defined as the process of adding together the assay value and levels of degradation products to see how closely these add up to 100 percent or not less than 95 % of the initial value, with due consideration of the margin of analytical precision.

Objective of Work

Rationale for choosing **"analytical method development & validation for cardiovascular & anti-diabetic drugs"** is that the cardiovascular disorders remain the most common cause of mortality and morbidity in the world and is an emerging threat. Cardiovascular disease continues to be the major disease causing health problems and responsible for majority of the deaths worldwide. Major drug administered areas for cardiovascular problems include heart failure, coronary artery diseases, blood clots, high cholesterol and others. India is among the top diabetic nations in the world. Major diabetes consequences include blindness, amputations, and kidney failure, with higher risk of death.

Now a days pharmaceutical counterfeiting is a world-wide emerging health problem, however it is more and more diffused in developing countries where drug-regulatory systems are weak and controls on production, distribution and import are inadequate. In India, the counterfeit medicines are stated to be 23 per cent of the market[9]. India is the largest manufacturer of generic drugs and probably 12-25% of the medicines supplied globally are contaminated, substandard and counterfeit[10]. The World Health Organization (WHO) defines a counterfeit medicine as that one which is deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with correct ingredients, wrong ingredients, without active ingredients, with incorrect quantity of active ingredients or with fake packaging.

Pharmaceutical industries are introducing more number of drugs into the market & number is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. There is a scope, therefore to develop newer analytical methods for such drugs.

Pharmaceutical industries are manufacturing effective single dose & multiple drug formulations to meet the market demand. For single dose or combination drugs, estimation of rapid, reliable & cost-effective analytical method is a challenging task for analytical method

researchers. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. In many methods, solvent selection to extraction of samples is time consuming. There is likely to be loss of accuracy and precision due to extractions and/or separation. The rapid analysis or simultaneous analysis procedures avoid these time consuming extractions and separations, and are economical in the sense use of expensive reagents is minimized. Thus, accurate, precise and efficient methods for rapid analysis for single dose or simultaneous estimation of the multicomponent formulations will be rational, as it will have immediate industrial applications.

So, to make the simple, robust and cost effective method for the single dose or simultaneous estimation of the combination made it worth while objective to perceive the present research work. It is also planned to validate the developed methods as per ICH norms

Plan of Work

1. Literature Survey

Literature survey will be done, available for listed single dose drugs and/or their combinations (regarding their physical and chemical properties, pharmacology and various analytical methods) shall be conducted.

This forms the basis for the development of new spectrophotometric, HPLC and HPTLC methods of analysis.

2. Procurement of Drug Sample

Procurement of drug specimens draws utmost priority. The drugs will be obtained either buying from open market or as gift sample from respective companies / research laboratories of those brands.

3. Development of Method

A. Using UV-Visible Spectrophotometer

1. Selection of suitable solvent having single component molecules & common solvent for combination drugs for quantitative extraction of the drug present in tablet formulation.

2. Selection of suitable method of analysis, depending upon spectral characteristics of the drug for single component molecules or simultaneous method of analysis for combination drugs.

- Selection of suitable sampling wavelength for that particular method chosen for analysis.
- Determination of appropriate working concentration range (depending upon the wavelength maximum or sampling wavelength selected) and number and composition of mixed standard suitable for the proposed method.
- Conformation of the developed method by authentic laboratory samples which resembles to the tablet sample and, by physical admixtures of varying proportion of the drug.

B. Using HPLC

- **Selection of solvent to be used as mobile phase**

For the phase, the first variable to be decide is whether an organic or aqueous eluent should be used with RP-HPLC analysis, either an aqueous eluent or a vary polar organic solvent such as methanol or acetonitrile should be tried first. If the K, values are too large with an aqueous solvent, then one of the solvent should be tried. If the K, values are too low the organic solvent, the separation should be attempted using a mixture of two solvent in various position, (K, = capacity factor, which is a measure of the degree where the peak of the interest is located with respect to void volume i.e. elution time of non-retained components (generally the values of K, is >2). If a buffer is used, the pH as well as the ionic strength of the buffer can be tried.

- **Selection of column**
  - Choose columns known to have long lifetimes at operating mobile phase pH.
  - Choose bonded phases on high purity, low acidity silica for best peak shape.
  - Select a C18 or C8 bonded phase first for good retention and resolution with typical acidic, basic and neutral samples.
  - If sample is expected to be difficult (i.e. very polar, difficult to retain, very basic) then select targeted bonded phases available for these types of samples.
  - The selected column should be capable to separate all degradant peaks from main peak and for its known impurities.

- **Sample solubility and stability.**

It is obvious that a solvent in which the sample to be analyzed is totally insoluble or incompatible with the solvent cannot be used as mobile phase.

A few milligrams of the sample placed in several vials, each filled with different chromatographic solvent, provide rapid visualization of solubility and stability, in the form precipitation, keeping it for define period of time.

For analytical purpose liquid most frequently used with RP-HPLC are tetrahydrofuran, acetonitrile, or methanol mixed with water and preferred mode is adsorption chromatography with silica as the stationary phase.

- **Mode of extraction**

All initial isolation of drug by means of extraction, filtration or other procedure is generally performed before the samples are chromatographed to separate the drug.

The extraction recovery can be estimated by comparing the slope of calibration curve based on calibration standard prepared according to the assay procedure.

Recovery should be 100%, however, recovery experiments are usually done when the assay method is being developed or validated.

- **Purity**

In liquid chromatography, it very important that both the sample and the solvent used in mobile phase be pure liquids, free of any solid particles; therefore, the sample solution should be filtered before injection. The solvent degassing known as purging or striping is done through the solution, which result in the elimination of gases.

Stationary phase selection depends on the number of factors such as characteristics and complexity of the sample and the selected mode of the separation.

- Study of nature of formulation, and possible measures to avoid inference of the excipients in the analysis of the combined dosage form.
- Simultaneous analysis of tablet formulation by the proposed method and the interference of the excipients in the analysis of the combined dosage form.

4. Validation of Method

Once the method is finalized it will be validated using a series of experiments designed to challenge the method and access its suitability to measure the analytes under normal running condition. The validation protocols usually followed will be that defined by the International Conference on Harmonization (ICH) under their validation for analytical procedure methodology, for following parameters,

- Specificity
- Linearity
- Range
- Accuracy
- Precision
- Detection limit
- Quantitation limit
- Robustness
- Ruggedness
- System suitable testing

5. Stability-Indicating Assay Method & Related substances method

Validated, quantitated analytical method will be assayed for stability & determine degradation impurities by related substances. so that the content of active ingredients, degradation products and the other component of interest can be accurately measured without interference.

The cardiovascular and antidiabetic drugs of single and multicomponent drug combinations will be taken for the investigation.

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